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Mechanical and in vitro performance of 13–93 bioactive glass scaffolds prepared by a polymer foam replication technique

Qiang Fu [a], Mohamed N. Rahaman [a,*], B. Sonny Bal [b], Roger F. Brown [c], Delbert E. Day [a,d]

[a] Department of Materials Science and Engineering, Missouri University of Science and Technology, Rolla, MO 65409, USA
[b] Department of Orthopaedic Surgery, University of Missouri-Columbia, Columbia, MO 65212, USA
[c] Department of Biological Sciences, Missouri University of Science and Technology, Rolla, MO 65409, USA
[d] Graduate Center for Materials Research, Missouri University of Science and Technology, Rolla, MO 65409, USA

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Abstract

A polymer foam replication technique was used to prepare porous scaffolds of 13–93 bioactive glass with a microstructure similar to that of human trabecular bone. The scaffolds, with a porosity of $85 \pm 2\%$ and pore size of 100–500 μm, had a compressive strength of 11 ± 1 MPa, and an elastic modulus of 3.0 ± 0.5 GPa, approximately equal to the highest values reported for human trabecular bone. The strength was also considerably higher than the values reported for polymeric, bioactive glass–ceramic and hydroxyapatite constructs prepared by the same technique and with the equivalent level of porosity. The in vitro bioactivity of the scaffolds was observed by the conversion of the glass surface to a nanostructured hydroxyapatite layer within 7 days in simulated body fluid at 37 °C. Protein and MTT assays of in vitro cell cultures showed an excellent ability of the scaffolds to support the proliferation of MC3T3-E1 preosteoblastic cells, both on the surface and in the interior of the porous constructs. Scanning electron microscopy showed cells with a closely adhering, well-spread morphology and a continuous increase in cell density on the scaffolds during 6 days of culture. The results indicate that the 13–93 bioactive glass scaffolds could be applied to bone repair and regeneration.

Keywords: Scaffold; Bioactive glass; Biomaterials; Cell culture; Tissue engineering

1. Introduction

The development of synthetic scaffolds and their processing into structures that have properties tailored for applications in bone repair and regeneration are becoming increasingly important, because of several shortcomings of autografts (limited supply and donor site morbidity) and allografts (immune rejection and possible transmission of pathogens). In addition to being biocompatible, scaffold materials for bone repair and regeneration should have adequate mechanical properties to support physiological loads. Tissue infiltration and facile integration of the scaffold with surrounding tissue are required for ultimate clinical application.

Some synthetic and natural polymers, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), copolymers of PLA and PGA, and collagen are biodegradable, so the scaffold can be gradually replaced by new bone matrix synthesized by tissue-forming cells [1–4]. However, the use of degradable polymers for replacing load-bearing bones is often challenging, because of their low mechanical strength [1]. Reinforcement with particles or short fibers of hydroxyapatite (HA) or bioactive glass improves the load-bearing properties of these polymers, and provides scaffolds that are biodegradable as well as bioactive [5–7].

Bioactive glasses, glass–ceramics and ceramics are attractive scaffold materials for bone repair, because of their abilities to enhance bone formation and to bond to surrounding tissue [8,9]. Upon implantation, bioactive glasses gradually convert to HA, the main mineral constituent of bone [9–12], and are osteoconductive as well as

* Corresponding author. Tel.: +1 573 341 4406; fax: +1 573 341 6934.
E-mail address: rahaman@mst.edu (M.N. Rahaman).

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osteoinductive [9]. Although brittle, bioactive glass scaffolds can provide higher mechanical strength than the aforementioned polymers [13]. The silicate-based bioactive glass designated 45S5, approved for in vivo use in the USA and elsewhere, has been widely investigated for biomedical applications [9]. The 45S5 glass cannot be easily pulled into fibers because of its tendency to devitrify (crystallize). Thermal bonding (sintering) of 45S5 particles into anatomically relevant shapes requires temperatures of ~1000 °C and higher, which leads to devitrification to form a predominantly combeite crystalline phase ($Na_2O \cdot 2CaO \cdot 3SiO_2$). While devitrification does not inhibit the ability to form an HA surface layer, the rate of conversion to HA (the bioactive potential) is reduced [14,15].

Another silicate-based bioactive glass, designated 13–93, with a modified 45S5 composition [16,17], has more facile viscous flow behavior and less tendency to crystallize than 45S5. The 13–93 glass is approved for in vivo use in Europe. The glass can be pulled into fibers, and particles or short fibers have been sintered, without devitrification, to form porous scaffolds with anatomically relevant shapes, such as the human proximal tibia [13]. Porous scaffolds consisting of 13–93 fiber rafts supported the in vitro growth and differentiation of MC3T3-E1 preosteoblastic cells [18]. Quantitative measurement of DNA showed no significant difference in cell proliferation between dense disks of 45S5 and 13–93 glass [18].

Several techniques have been employed to produce porous three-dimensional scaffolds of polymers and bioactive ceramics. These methods include thermally induced phase separation (TIPS) [19,20], solid freeform fabrication [21,22], solvent casting and particle leaching [23,24], freeze-casting [25–28] and polymer foam replication [15,29–32]. Interconnected pores with a mean diameter (or width) of 100 μm or greater and open porosity of >50% are generally considered to be the minimum requirements to permit tissue ingrowth and function in porous scaffolds [33,34].

Using a foam with the appropriate architecture, scaffolds with a microstructure approximating trabecular bone can be prepared by the polymer foam infiltration technique [35]. In this technique, a polymer foam is infiltrated with a stable suspension of colloidal particles. After drying, the system is heated to decompose the polymer foam, and sintered at a higher temperature to densify the network of particles. The method has been used to prepare porous scaffolds of 45S5 glass–ceramic [15], HA [29,30], biphasic calcium phosphate [31] and akermanite [32]. The strength of the construct is critically dependent on the ability to achieve a solid network with high density. This is dependent on the particle packing of the infiltrated foam, which, in turn, is dependent on the colloid stability of the suspension used to infiltrate the foam. Generally, a stable suspension leads to a more homogeneous and higher particle packing density in the infiltrated foam, which leads to more facile densification of the particulate network.

Based on the aforementioned forming characteristics and bioactivity of 13–93 glass, coupled with the ability of the polymer foam infiltration technique to produce a bone-like microstructure, an investigation of the mechanical and in vitro performance of 13–93 glass scaffolds prepared by this technique was undertaken. The microstructure and compressive mechanical properties of the fabricated scaffolds were characterized, and the ability of the constructs to support the attachment and growth of osteoblastic cells was evaluated. The mouse MC3T3-E1 cell line chosen for these experiments has been used extensively in previous in vitro investigations of biomaterials for bone repair and tissue engineering [36,37].

2. Materials and methods

2.1. Preparation of 13–93 glass scaffolds

Glass with the 13–93 composition (wt.%) ($53SiO_2$, $6Na_2O$, $12K_2O$, 5MgO, CaO, $4P_2O_5$) was prepared by melting a mixture of analytical grade Na_2CO_3, K_2CO_3, $MgCO_3$, $CaCO_3$, SiO_2 and $NaH_2PO_4 \cdot 2H_2O$ (Fisher Scientific, St. Louis, MO) in a platinum crucible at 1300 °C and quenching between stainless steel plates. The glass was crushed in a hardened steel mortar and pestle and classified using stainless steel sieves to provide particles of size <150 μm. These particles were further ground for 2 h in an attrition mill (Model 01-HD, Union Process, Akron, OH), using high-purity Y_2O_3-stabilized ZrO_2 milling media and ethanol as the solvent, to provide particles in the colloidal size range (<5–10 μm). The average size and size distribution of the particles were measured using a laser diffraction particle size analyzer (Model LS 13 320; Beckman Coulter Inc., Fullerton, CA).

The colloidal properties of the 13–93 glass particles and the ability of different dispersants to stabilize the particles in water were investigated in order to prepare stable suspensions for use in the polymer foam replication technique. Preliminary experiments were performed to test the sedimentation behavior of suspensions (5 vol.% particles) stabilized with 0.25–2 wt.% of five different dispersants (based on the dry mass of the particles). The dispersants were ammonium polymethacrylate (Darvan C; mol. wt. = 10,000–16,000; R.T. Vanderbilt Co., Norwalk, CT), sodium polyacrylate (Darvan 811, mol. wt. = 5000; R.T. Vanderbilt Co.), poly(methylvinyl ether) (EasySperse; ISP, Wayne, NJ), Dynol 604 (Air Products & Chemicals Inc., Allentown, PA) and Targon 1128 (BK Ladenburg GmbH, Ladenburg, Germany). The suspensions were poured to a height of ~5 cm into test tubes (~3 cm in diameter × 6.5 cm) and, after vigorous shaking, allowed to settle for 24 h. The suspensions stabilized with Dynol 604 or Targon 1128 settled almost completely to give loose flocculated sediments, indicating that these two dispersants were ineffective for stabilizing the particles. On the other hand, the suspensions stabilized with EasySperse showed the least sedimentation, whereas Darvan C or Darvan 811 produced sedimentation results that were intermediate between those for EasySperse and Dynol 604 (or Targon 1128). Because of

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